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Cloning of mlCNBD-FRET

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An abbreviated version of this protocol was published in eLIFE in Mar 2016

A novel biosensor to study cAMP dynamics in cilia and flagella

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Detailed protocol

The mlCNBD cDNA sequence (cyclic nucleotide binding-domain) of the cyclic nucleotide-gated K+ channel from Mesorhizobium loti (MAFF303099, mll3241) was amplified via PCR (Cukkemane et al.,2007) using the following primers:

- C0686: 5'- GGCGGATCCCAAGAAGTCCGTCGCGG-3'
- C0687: 5'- GCCTCGAGCGCTCGCCGCAGCG-3'

For expression in mammalian cell lines, citrine and cerulean were amplified by PCR and fused to the N- and C-terminus of the mlCNBD via BamHI/HindIII or XhoI/ApaI, respectively.

- citrine: C0053/0089 (BamHI/HindIII)
- C0053: 5'-CCCAAGCTTCCACCATGGTGAGCAAGGGCGAGGAG-3'
- C0089: 5'-CGGGATCCCTTGTACAGCTCGTCCATGCCGAGAGTGATCCC-3'
- cerulean: C0057/58 (XhoI/ApaI)
- C0057: 5'-CCGCTCGAGATGGTGAGCAAGGGCGAG-3'
- C0058: 5'-GCAGGGCCCTAGTGATGGTGATGGTGATGATGGTGATGATGC-3'

The C-termi-nus of cerulean contained a histidine (His10) tag. The PCR product was cloned into a pcDNA3.1(+) vector (Invitrogen, Darmstadt, Germany) using BamHI and ApaI (pc3.1-mlCNBD-FRET).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Wachten, D. (2021). Cloning of mlCNBD-FRET. Bio-protocol Preprint. bio-protocol.org/prep844.
2. Mukherjee, S., Jansen, V., Jikeli, J. F., Hamzeh, H., Alvarez, L., Dombrowski, M., Balbach, M., Strünker, T., Seifert, R., Kaupp, U. B. and Wachten, D.(2016). A novel biosensor to study cAMP dynamics in cilia and flagella. eLIFE. DOI: 10.7554/eLife.14052